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(54) Abstract Title
Voltage-gated potassium channel polypeptides

(57) The present invention provides an isolated voltage-gated potassium channel polypeptide comprising
(i) the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4; or
(ii) a variant thereof which capable of forming a channel which can be activated by depolarisation of the cell membrane potential above the reversal potential for K^+ (E_K); or
(iii) a fragment of (i) or (ii) which capable of forming a channel which can be activated by depolarisation of the cell membrane potential above the reversal potential for K^+ (E_K).

The polypeptide may be used in a method to identify substances that may be useful in the treatment of pain alzheimers disease, epilepsy, psychiatric disorders and thyroid disorders.

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(58) Field of Search
Online:EPODOC, WPI, PAJ, BIOSIS, CAPLUS, EMBASE, MEDLINE, SCISEARCH, BLASTp, BLASTn

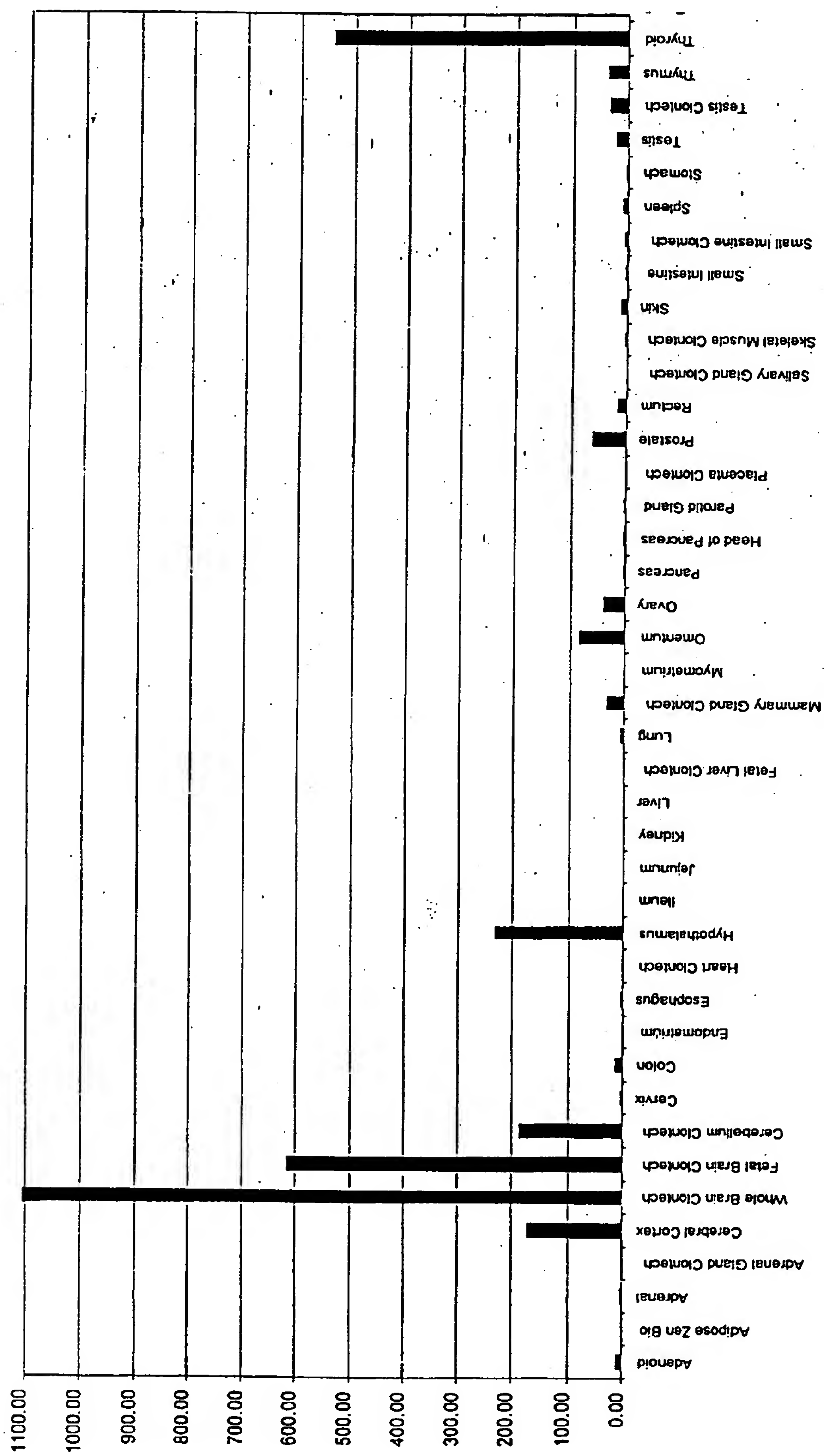
Adenoid
Adipose Zen Bio
Adrenal
Adrenal Gland Clontech
Cerebral Cortex
Whole Brain Clontech
Fetal Brain Clontech
Cerebellum Clontech
Cervix
Colon
Endometrium
Esophagus
Heart Clontech
Hypothalamus
Ileum
Jejunum
Kidney
Liver
Fetal Liver Clontech
Lung
Mammary Gland Clontech
Myometrium
Omentum
Ovary
Pancreas
Head of Pancreas
Parotid Gland
Placenta Clontech
Prostate
Rectum
Salivary Gland Clontech
Skeletal Muscle Clontech
Skin
Small Intestine
Small Intestine Clontech
Spleen
Stomach
Testis
Testis Clontech
Thymus
Thyroid
0.00
100.00
200.00
300.00
400.00
500.00
600.00
700.00
800.00
900.00
1000.00
1100.00

Figure 1

NEW PROTEIN

Field of the Invention

The present invention relates to voltage-gated potassium channel polypeptides.

Background of the Invention

Ion channels are involved in a wide variety of neurological and other disorders in man. Voltage-gated potassium channels of the Kv3 family yield delayed rectifier type currents when expressed in heterologous expression systems.

Kv3 channel subtypes have high activation voltage and fast deactivation rates which help repolarise action potentials rapidly without subsequently influencing the action potential generation threshold. The rapid deactivation of Kv3 channel currents leads to a fast recovering afterhyperpolarisation which maximises recovery of sodium channels from inactivation. Thus the fast recovering afterhyperpolarisation is one factor that enables Kv3 expressing neurons to fire at high frequencies and to regulate synaptic transmission. Presynaptic voltage-gated potassium channels affect Ca^{2+} entry and neurotransmitter release.

Summary of the Invention

A novel voltage-gated potassium channel, referred to herein as HIPHUM 59/60/190, is now provided. HIPHUM 59/60/190 is shown to be primarily expressed in brain (whole brain, fetal brain, cerebral cortex, cerebellum, hypothalamus) and thyroid. The novel voltage-gated potassium channel is a screening target for the identification and development of novel pharmaceutical agents, including modulators of voltage-gated potassium channel activity. These agents may be used in the treatment and/or prophylaxis of disorders such as epilepsy, juvenile myoclonic epilepsy (JME), temporal lobe epilepsy (TLE), seizure disorders, sleep disorders such as insomnia, hypersomnia, parasomnia, sleep apnea syndromes and stupor, pain states such as acute postoperative pain, psychogenic pain syndromes, pain from cancer, glossopharyngeal neuralgia, inflammatory pain, neuropathic pain, migraine, trigeminal neuralgia, headache and tension headache, neurodegenerative diseases

such as Alzheimer's disease, Huntington's disease, Parkinson's disease, palsies and paralysis, pyschiatric disorders such as anxiety, depression, bipolar disorder, schizophrenia and paranoid psychoses and thyroid disorders such as euthyroid sick syndrome, hyperthyroidism, hypothyroidism, simple goiter and thyroiditis.

Accordingly, the present invention provides an isolated voltage-gated potassium channel polypeptide comprising:

(i) the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4;

(ii) a variant thereof which capable of forming a channel which can be activated by depolarisation of the cell membrane potential above the reversal potential for K^+ (E_K); or

(iii) a fragment of (i) or (ii) which capable of forming a channel which can be activated by depolarisation of the cell membrane potential above the reversal potential for K^+ (E_K).

According to another aspect of the invention there is provided a polynucleotide encoding a polypeptide of the invention which polynucleotide includes a sequence comprising:

(a) the nucleic acid sequence of SEQ ID NO: 1 or SEQ ID NO: 3 and/or a sequence complementary thereto;

(b) a sequence which hybridises under stringent conditions to a sequence as defined in (a);

(c) a sequence that is degenerate as a result of the genetic code to a sequence as defined in (a) or (b); or

(d) a sequence having at least 98% identity to a sequence as defined in (a), (b) or (c).

The invention also provides:

- an expression vector which comprises a polynucleotide of the invention and which is capable of expressing a polypeptide of the invention;
- a host cell comprising an expression vector of the invention;
- a method of producing a polypeptide of the invention which method comprises maintaining a host cell of the invention under conditions suitable for obtaining expression of the polypeptide and isolating the said polypeptide;
- an antibody specific for a polypeptide of the invention;

- a method for identification of a substance that modulates voltage-gated potassium channel activity and/or expression, which method comprises contacting a polypeptide, polynucleotide, expression vector or host cell of the invention with a test substance and determining the effect of the test substance on the activity and/or expression of the said polypeptide or the polypeptide encoded by the said polynucleotide, thereby to determine whether the test substance modulates voltage-gated potassium channel activity and/or expression;
- a compound which stimulates or modulates voltage-gated potassium channel activity and which is identifiable by the method referred to above;
- a method of treating a subject having a disorder that is responsive to voltage-gated potassium channel stimulation or modulation, which method comprises administering to said subject an effective amount of substance of the invention; and
- use of a substance that stimulates or modulates voltage-gated potassium channel activity in the manufacture of a medicament for the treatment or prophylaxis of a disorder that is responsive to stimulation or modulation of voltage-gated potassium channel activity.

Preferably the disorder is selected from epilepsy, juvenile myoclonic epilepsy (JME), temporal lobe epilepsy (TLE), seizure disorders, sleep disorders such as insomnia, hypersomnia, parasomnia, sleep apnea syndromes and stupor, pain states such as acute postoperative pain, psychogenic pain syndromes, pain from cancer, glossopharyngeal neuralgia, inflammatory pain, neuropathic pain, migraine, trigeminal neuralgia, headache and tension headache, neurodegenerative diseases such as Alzheimer's disease, Huntington's disease, Parkinson's disease, palsies and paralysis, pyschiatric disorders such as anxiety, depression, bipolar disorder, schizophrenia and paranoid psychoses and thyroid disorders such as euthyroid sick syndrome, hyperthyroidism, hypothyroidism, simple goiter and thyroiditis.

Brief Description of the Figures

Figure 1 shows the relative expression levels of HIPHUM 59/60/190 in a variety of human tissues.

Brief Description of the Sequences

SEQ ID NO: 1 shows the nucleotide and amino acid sequences of the longer splice variant of human protein HIPHUM 59/60/190.

SEQ ID NO: 2 is the amino acid sequence alone of the longer splice variant of HIPHUM 59/60/190.

SEQ ID NO: 3 shows the nucleotide and amino acid sequences of the shorter splice variant of human protein HIPHUM 59/60/190.

SEQ ID NO: 4 is the amino acid sequence alone of the shorter splice variant of HIPHUM 59/60/190.

Detailed Description of the Invention

Throughout the present specification and the accompanying claims the words "comprise" and "include" and variations such as "comprises", "comprising", "includes" and "including" are to be interpreted inclusively. That is, these words are intended to convey the possible inclusion of other elements or integers not specifically recited, where the context allows.

The present invention relates to a human voltage-gated potassium channel, referred to herein as HIPHUM 59/60/190, and variants thereof. Sequence information for HIPHUM 59/60/190 is provided in SEQ ID NO: 1 and SEQ ID NO: 3 (nucleotide and amino acid) and in SEQ ID NO: 2 and SEQ ID NO: 4 (amino acid). A polypeptide of the invention thus consists essentially of the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4 or of a variant of either sequence, or of a fragment of any thereof.

Polypeptides of the invention may be in a substantially isolated form. It will be understood that the polypeptide may be mixed with carriers or diluents which will not interfere with the intended purpose of the polypeptide and still be regarded as substantially isolated. A polypeptide of the invention may also be in a substantially purified form, in which case it will generally comprise the polypeptide in a preparation in which more than 50%, e.g. more than 80%, 90%, 95% or 99%, by weight of the polypeptide in the preparation is a polypeptide of the invention. Routine methods, can be employed to purify and/or synthesise the proteins according

to the invention. Such methods are well understood by persons skilled in the art, and include techniques such as those disclosed in Sambrook *et al*, Molecular Cloning: a Laboratory Manual, 2nd Edition, CSH Laboratory Press, 1989, the disclosure of which is included herein in its entirety by way of reference.

The term "variant" refers to a polypeptide which has a same essential character or basic biological functionality as HIPHUM 59/60/190. The essential character of HIPHUM 59/60/190 can be defined as follows: HIPHUM 59/60/190 is a voltage-gated potassium channel. Preferably the polypeptide is capable of forming a delayed-rectifier potassium channel which can be activated by depolarisation of the cell membrane potential above the reversal potential for K^+ (E_K). Preferably a variant polypeptide is one which binds to the same the same Kv3 subfamily members as HIPHUM 59/60/190. Preferably the channel is inhibited by phosphorylation for example, mediated by PKA, PKC or PKG. Preferably, a variant of the longer splice variant of HIPHUM 59/60/190 contains a PKC phosphorylation site. Preferably, a variant of either the splice variant of HIPHUM 59/60/190 contains a PKA phosphorylation site. A polypeptide having the same essential character as HIPHUM 59/60/190 may be identified by monitoring for potassium channel activity of the voltage-gated potassium channel. Potassium channel activity may be monitored electrophysiologically, for example by monitoring the threshold for channel activation, the rate of inactivation, the rate of recovery from inactivation or the rate of deactivation. Alternatively a conformational change in the potassium channel or changes in intracellular K^+ and/or Rb^+ ion concentration may be monitored.

In another aspect of the invention, a variant is one which does not show the same activity as HIPHUM 59/60/190 but is one which inhibits or enhances a basic function of HIPHUM 59/60/190. For example, a variant polypeptide is one which inhibits formation of potassium channels by binding to HIPHUM 59/60/190 or another Kv3 subfamily member to prevent homomeric or heteromeric channel assembly. A variant polypeptide that enhances channel activity may lack one or more consensus phosphorylation site. A variant of HIPHUM 59/60/190 that lacks a consensus phosphorylation site will show a decreased sensitivity to inactivation by phosphorylation.

Typically, polypeptides with more than about 98% identity preferably at least

99% and particularly preferably at least 99.5% identity, with the amino acid sequences of SEQ ID NO: 2 or SEQ ID NO: 4, are considered as variants of the proteins. Such variants may include allelic variants and the deletion, modification or addition of single amino acids or groups of amino acids within the protein sequence, as long as the peptide maintains a basic biological functionality of HIPHUM 59/60/190.

Amino acid substitutions may be made, for example from 1, 2 or 3 to 10, 20 or 30 substitutions. The modified polypeptide generally retains activity as a voltage-gated potassium channel. Conservative substitutions may be made, for example according to the following Table. Amino acids in the same block in the second column and preferably in the same line in the third column may be substituted for each other.

ALIPHATIC	Non-polar	G A P
		I L V
	Polar-uncharged	C S T M
		N Q
	Polar-charged	D E
		K R
AROMATIC		H F W Y

Shorter polypeptide sequences are within the scope of the invention. For example, a peptide of at least 20 amino acids or up to 50, 60, 70, 80, 100, 150, 200,

300, 400 or 500 amino acids in length is considered to fall within the scope of the invention as long as it demonstrates a basic biological functionality of HIPHUM 59/60/190. In particular, but not exclusively, this aspect of the invention encompasses the situation when the protein is a fragment of the complete protein sequence and may represent a Kv3 polypeptide-binding region. Such fragments can be used to construct chimeric receptors preferably with another voltage-gated potassium channel polypeptide, more preferably with another delayed rectifier voltage-gated potassium channel, such as a Kv3 polypeptide. Such fragments of HIPHUM 59/60/190 or a variant thereof can also be used to raise anti-HIPHUM 59/60/190 antibodies. In this embodiment the fragment may comprise an epitope of the HIPHUM 59/60/190 polypeptide and may otherwise not demonstrate the properties of HIPHUM 59/60/190, such as the ability to form functional potassium channels. A preferred fragment comprises the amino acid sequence from positions 42 to 92 of SEQ ID NO: 2 or SEQ ID NO: 4. Further preferred fragments comprise a fragment of SEQ ID NO: 2 or SEQ ID NO: 4 which includes the amino acids at positions 186 to 187, position 502, position 210, position 270 and/or position 529 of SEQ ID NO: 2 or SEQ ID NO: 4.

Polypeptides of the invention may be chemically modified, e.g. post-translationally modified. For example, they may be glycosylated or comprise modified amino acid residues. They may also be modified by the addition of histidine residues to assist their purification or by the addition of a signal sequence to promote insertion into the cell membrane. Such modified polypeptides fall within the scope of the term "polypeptide" of the invention.

The invention also includes nucleotide sequences that encode for HIPHUM 59/60/190 or variants thereof as well as nucleotide sequences which are complementary thereto. The nucleotide sequence may be RNA or DNA including genomic DNA, synthetic DNA or cDNA. Preferably the nucleotide sequence is a DNA sequence and most preferably, a cDNA sequence. Nucleotide sequence information is provided in SEQ ID NO: 1 and SEQ ID NO: 3. Such nucleotides can be isolated from human cells or synthesised according to methods well known in the art, as described by way of example in Sambrook *et al*, 1989.

Typically a polynucleotide of the invention comprises a contiguous sequence

of nucleotides which is capable of hybridizing under selective conditions to the coding sequence or the complement of the coding sequence of SEQ ID NO: 1 or SEQ ID NO: 3.

A polynucleotide of the invention can hydridize to the coding sequence or the complement of the coding sequence of SEQ ID NO: 1 or SEQ ID NO: 3 at a level significantly above background. Background hybridization may occur, for example, because of other cDNAs present in a cDNA library. The signal level generated by the interaction between a polynucleotide of the invention and the coding sequence or complement of the coding sequence of SEQ ID NO: 1 or SEQ ID NO: 3 is typically at least 10 fold, preferably at least 100 fold, as intense as interactions between other polynucleotides and the coding sequence of SEQ ID NO: 1 or SEQ ID NO: 3. The intensity of interaction may be measured, for example, by radiolabelling the probe, e.g. with ^{32}P. Selective hybridisation may typically be achieved using conditions of medium to high stringency. However, such hybridisation may be carried out under any suitable conditions known in the art (see Sambrook *et al*, 1989. For example, if high stringency is required suitable conditions include from 0.1 to 0.2 x SSC at 60 °C up to 65 °C. If lower stringency is required suitable conditions include 2 x SSC at 60 °C.

The coding sequence of SEQ ID NO: 1 or SEQ ID NO: 3 may be modified by nucleotide substitutions, for example from 1, 2 or 3 to 10, 25, 50 or 100 substitutions. The polynucleotide of SEQ ID NO: 1 or SEQ ID NO: 3 may alternatively or additionally be modified by one or more insertions and/or deletions and/or by an extension at either or both ends. A polynucleotide may include one or more introns, for example may comprise genomic DNA. Additional sequences such as signal sequences which may assist in insertion of the polypeptide in a cell membrane may also be included. The modified polynucleotide generally encodes a polypeptide which has HIPHUM 59/60/190 activity. Alternatively, a polynucleotide encodes a portion of a polypeptide or a polypeptide which inhibits HIPHUM 59/60/190 activity, for example by disrupting the formation of channels containing HIPHUM 59/60/190 by binding HIPHUM 59/60/190 to prevent homomeric channel assembly. Degenerate substitutions may be made and/or substitutions may be made which would result in a conservative amino acid substitution when the modified sequence is

translated, for example as shown in the Table above.

A nucleotide sequence which is capable of selectively hybridizing to the complement of the DNA coding sequence of SEQ ID NO: 1 or SEQ ID NO: 3 will generally have at least at least 98%, at least 99% or at least 99.5% sequence identity to the coding sequence of SEQ ID NO: 1 or SEQ ID NO: 3 over a region of at least 20, preferably at least 30, for instance at least 40, at least 60, at least 100, at least 200, at least 500, more preferably at least 1000 contiguous nucleotides or most preferably over the full length of SEQ ID NO: 1 or SEQ ID NO: 3.

For example the UWGCG Package provides the BESTFIT program which can be used to calculate homology (for example used on its default settings) (Devereux *et al* (1984) *Nucleic Acids Research* **12**, p387-395). The PILEUP and BLAST algorithms can be used to calculate homology or line up sequences (typically on their default settings), for example as described in Altschul (1993) J. Mol. Evol. 36:290-300; Altschul *et al* (1990) J. Mol. Biol. 215:403-10.

Software for performing BLAST analyses is publicly available through the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pair (HSPs) by identifying short words of length W in the query sequence that either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighbourhood word score threshold (Altschul *et al*, 1990). These initial neighbourhood word hits act as seeds for initiating searches to find HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extensions for the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a word length (W) of 11, the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1992) *Proc. Natl. Acad. Sci.* USA 89: 10915-10919) alignments (B) of 50, expectation (E) of 10, M=5, N=4, and a comparison of both strands.

The BLAST algorithm performs a statistical analysis of the similarity between two sequences; see e.g., Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90: 5873-5787. One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a sequence is considered similar to another sequence if the smallest sum probability in comparison of the first sequence to the second sequence is less than about 1, preferably less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

Any combination of the above mentioned degrees of sequence identity and minimum sizes may be used to define polynucleotides of the invention, with the more stringent combinations (i.e. higher sequence identity over longer lengths) being preferred. Thus, for example a polynucleotide which has at least 98% sequence identity over 25, preferably over 30 nucleotides forms one aspect of the invention, as does a polynucleotide which has at least 99% sequence identity over 40 nucleotides.

The nucleotides according to the invention have utility in production of the proteins according to the invention, which may take place *in vitro, in vivo* or *ex vivo*. The nucleotides may be involved in recombinant protein synthesis or indeed as therapeutic agents in their own right, utilised in gene therapy techniques. Nucleotides complementary to those encoding HIPHUM 59/60/190, or antisense sequences, may also be used in gene therapy.

Polynucleotides of the invention may be used as a primer, e.g. a PCR primer, a primer for an alternative amplification reaction, a probe e.g. labelled with a revealing label by conventional means using radioactive or non-radioactive labels, or the polynucleotides may be cloned into vectors.

Such primers, probes and other fragments will preferably be at least 10, preferably at least 15 or at least 20, for example at least 25, at least 30 or at least 40 nucleotides in length. They will typically be up to 40, 50, 60, 70, 100 or 150 nucleotides in length. Probes and fragments can be longer than 150 nucleotides in length, for example up to 200, 300, 400, 500, 600, 700, 1000, 1500 or 2000 nucleotides in length, or even up to a few nucleotides, such as five or ten nucleotides, short of the coding sequence of SEQ ID NO: 1 or SEQ ID NO: 3.

The present invention also includes expression vectors that comprise nucleotide sequences encoding the proteins or variants thereof of the invention. Such expression vectors are routinely constructed in the art of molecular biology and may for example involve the use of plasmid DNA and appropriate initiators, promoters, enhancers and other elements, such as for example polyadenylation signals which may be necessary, and which are positioned in the correct orientation, in order to allow for protein expression. Other suitable vectors would be apparent to persons skilled in the art. By way of further example in this regard we refer to Sambrook *et al.* 1989.

Polynucleotides according to the invention may also be inserted into the vectors described above in an antisense orientation in order to provide for the production of antisense RNA. Antisense RNA or other antisense polynucleotides may also be produced by synthetic means. Such antisense polynucleotides may be used as test compounds in the assays of the invention or may be useful in a method of treatment of the human or animal body by therapy.

Preferably, a polynucleotide of the invention or for use in the invention in a vector is operably linked to a control sequence which is capable of providing for the expression of the coding sequence by the host cell, i.e. the vector is an expression vector. The term "operably linked" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. A regulatory sequence, such as a promoter, "operably linked" to a coding sequence is positioned in such a way that expression of the coding sequence is achieved under conditions compatible with the regulatory sequence.

The vectors may be for example, plasmid, virus or phage vectors provided with a origin of replication, optionally a promoter for the expression of the said polynucleotide and optionally a regulator of the promoter. The vectors may contain one or more selectable marker genes, for example an ampicillin resistence gene in the case of a bacterial plasmid or a resistance gene for a fungal vector. Vectors may be used *in vitro*, for example for the production of DNA or RNA or used to transfect or transform a host cell, for example, a mammalian host cell. The vectors may also be adapted to be used *in vivo*, for example in a method of gene therapy.

Promoters and other expression regulation signals may be selected to be

compatible with the host cell for which expression is designed. For example, yeast promoters include *S. cerevisiae* GAL4 and ADH promoters, *S. pombe nmt*1 and *adh* promoter. Mammalian promoters include the metallothionein promoter which can be induced in response to heavy metals such as cadmium. Viral promoters such as the SV40 large T antigen promoter or adenovirus promoters may also be used. All these promoters are readily available in the art.

Mammalian promoters, such as β-actin promoters, may be used. Tissue-specific promoters are especially preferred. Viral promoters may also be used, for example the Moloney murine leukaemia virus long terminal repeat (MMLV LTR), the rous sarcoma virus (RSV) LTR promoter, the SV40 promoter, the human cytomegalovirus (CMV) IE promoter, adenovirus, HSV promoters (such as the HSV IE promoters), or HPV promoters, particularly the HPV upstream regulatory region (URR). Viral promoters are readily available in the art.

The vector may further include sequences flanking the polynucleotide giving rise to polynucleotides which comprise sequences homologous to eukaryotic genomic sequences, preferably mammalian genomic sequences, or viral genomic sequences. This will allow the introduction of the polynucleotides of the invention into the genome of eukaryotic cells or viruses by homologous recombination. In particular, a plasmid vector comprising the expression cassette flanked by viral sequences can be used to prepare a viral vector suitable for delivering the polynucleotides of the invention to a mammalian cell. Other examples of suitable viral vectors include herpes simplex viral vectors and retroviruses, including lentiviruses, adenoviruses, adeno-associated viruses and HPV viruses. Gene transfer techniques using these viruses are known to those skilled in the art. Retrovirus vectors for example may be used to stably integrate the polynucleotide giving rise to the polynucleotide into the host genome. Replication-defective adenovirus vectors by contrast remain episomal and therefore allow transient expression.

The invention also includes cells that have been modified to express the HIPHUM 59/60/190 polypeptide or a variant thereof. Such cells include transient, or preferably stable higher eukaryotic cell lines, such as mammalian cells or insect cells, using for example a baculovirus expression system, lower eukaryotic cells, such as yeast or prokaryotic cells such as bacterial cells. Particular examples of cells which

may be modified by insertion of vectors encoding for a polypeptide according to the invention include mammalian HEK293T, CHO, HeLa, BHK, 3T3 and COS cells. Preferably the cell line selected will be one which is not only stable, but also allows for mature glycosylation and cell surface expression of a polypeptide. Expression may be achieved in transformed oocytes. A polypeptide of the invention may be expressed in cells of a transgenic non-human animal, preferably a mouse. A transgenic non-human animal expressing a polypeptide of the invention is included within the scope of the invention. A polypeptide of the invention may also be expressed in *Xenopus laevis* oocytes, in particular for use in an assay of the invention. A polypeptide of the invention may be purified from any suitable cell type from any species for reconstitution into lipid bilayers or vesicles.

According to another aspect, the present invention also relates to antibodies, specific for a polypeptide of the invention. Such antibodies are for example useful in purification, isolation or screening methods involving immunoprecipitation techniques or, indeed, as therapeutic agents in their own right.

Antibodies may be raised against specific epitopes of the polypeptides according to the invention. Such antibodies may be used to block the same Kv3 subfamily members binding to the polypeptide. An antibody, or other compound, "specifically binds" to a protein when it binds with preferential or high affinity to the protein for which it is specific but does substantially bind not bind or binds with only low affinity to other proteins. A variety of protocols for competitive binding or immunoradiometric assays to determine the specific binding capability of an antibody are well known in the art (see for example Maddox *et al*, J. Exp. Med. 158, 1211-1226, 1993). Such immunoassays typically involve the formation of complexes between the specific protein and its antibody and the measurement of complex formation.

Antibodies of the invention may be antibodies to human polypeptides or fragments thereof. For the purposes of this invention, the term "antibody", unless specified to the contrary, includes fragments which bind a polypeptide of the invention. Such fragments include Fv, F(ab') and $F(ab')_2$ fragments, as well as single chain antibodies. Furthermore, the antibodies and fragment thereof may be chimeric antibodies, CDR-grafted antibodies or humanised antibodies.

Antibodies may be used in a method for detecting polypeptides of the invention in a biological sample, which method comprises:

I providing an antibody of the invention;

II incubating a biological sample with said antibody under conditions which allow for the formation of an antibody-antigen complex; and

III determining whether antibody-antigen complex comprising said antibody is formed.

A sample may be for example a tissue extract, blood, serum and saliva. Antibodies of the invention may be bound to a solid support and/or packaged into kits in a suitable container along with suitable reagents, controls, instructions, etc. Antibodies may be linked to a revealing label and thus may be suitable for use in methods of *in vivo* HIPHUM 59/60/190 imaging.

Antibodies of the invention can be produced by any suitable method. Means for preparing and characterising antibodies are well known in the art, see for example Harlow and Lane (1988) "Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. For example, an antibody may be produced by raising antibody in a host animal against the whole polypeptide or a fragment thereof, for example an antigenic epitope thereof, herein after the "immunogen".

A method for producing a polyclonal antibody comprises immunising a suitable host animal, for example an experimental animal, with the immunogen and isolating immunoglobulins from the animal's serum. The animal may therefore be inoculated with the immunogen, blood subsequently removed from the animal and the IgG fraction purified.

A method for producing a monoclonal antibody comprises immortalising cells which produce the desired antibody. Hybridoma cells may be produced by fusing spleen cells from an inoculated experimental animal with tumour cells (Kohler and Milstein (1975) *Nature* 256, 495-497).

An immortalized cell producing the desired antibody may be selected by a conventional procedure. The hybridomas may be grown in culture or injected intraperitoneally for formation of ascites fluid or into the blood stream of an allogenic host or immunocompromised host. Human antibody may be prepared by *in*

vitro immunisation of human lymphocytes, followed by transformation of the lymphocytes with Epstein-Barr virus.

For the production of both monoclonal and polyclonal antibodies, the experimental animal is suitably a goat, rabbit, rat or mouse. If desired, the immunogen may be administered as a conjugate in which the immunogen is coupled, for example via a side chain of one of the amino acid residues, to a suitable carrier. The carrier molecule is typically a physiologically acceptable carrier. The antibody obtained may be isolated and, if desired, purified.

An important aspect of the present invention is the use of polypeptides according to the invention in screening methods. The screening methods may be used to identify substances that bind to voltage-gated potassium channels and in particular which bind to HIPHUM 59/60/190. Screening methods may also be used to identify agonists or antagonists which may modulate voltage-gated potassium channel activity, inhibitors or activators of HIPHUM 59/60/190 activity, and/or agents which up-regulate or down-regulate HIPHUM 59/60/190 expression.

Any suitable format may be used for the assay. In general terms such screening methods may involve contacting a polypeptide of the invention with a test substance and monitoring for binding of the test substance to the polypeptide or measuring receptor activity. A polypeptide of the invention may be incubated with a test substance. Modulation of voltage-gated potassium channel activity may be determined. In a preferred aspect, the assay is a cell-based assay. Preferably the assay may be carried out in a single well of a microtitre plate. Assay formats which allow high throughput screening are preferred.

Modulator activity can be determined by contacting cells expressing a polypeptide of the invention with a substance under investigation and by monitoring an effect mediated by the polypeptide. The cells expressing the polypeptide may be *in vitro* or *in vivo*. The polypeptide of the invention may be naturally or recombinantly expressed. Preferably, the assay is carried out *in vitro* using cells expressing recombinant polypeptide. Preferably, control experiments are carried out on cells which do not express the polypeptide of the invention to establish whether the observed responses are the result of activation of the polypeptide. Typically the cells will express other Kv3 subfamily members.

The binding of a test substance to a polypeptide of the invention can be determined directly. For example, a radiolabelled test substance can be incubated with the polypeptide of the invention and binding of the test substance to the polypeptide can be monitored. Typically, the radiolabelled test substance can be incubated with cell membranes containing the polypeptide until equilibrium is reached. The membranes can then be separated from a non-bound test substance and dissolved in scintillation fluid to allow the radioactive content to be determined by scintillation counting. Non-specific binding of the test substance may also be determined by carrying out a competitive binding assay.

Substances that inhibit the interaction of a polypeptide of the invention with other Kv3 subfamily members may also be identified through a yeast 2-hybrid assay or other protein interaction assay such as a co-immunoprecipitation or an ELISA based technique.

Assays may be carried out using cells expressing HIPHUM 59/60/190, and optionally other Kv3 subfamily members, and incubating such cells with the test substance. The results of the assay are compared to the results obtained using the same assay in the absence of the test substance. Cells expressing HIPHUM 59/60/190 constitutively may be provided for use in assays for HIPHUM 59/60/190 function. Additional test substances may be introduced in any assay to look for inhibitors or enhancers of HIPHUM 59/60/190-mediated activity, preferably delayed rectifier potassium channel activity.

The ability of a test substance to modulate the HIPHUM 59/60/190 regulated flow of potassium ions through voltage-gated potassium channels may also be determined using fluorescence based assays using a Fluorometric Imaging Plate Reader (FLIPR) and membrane voltage sensitive dyes, such as DiBac, or K^+/Rb^+ sensitive dyes. FRET/BRET based membrane voltage sensitive dyes with VIPR may also be used.

Assays may also be carried out by measuring the influx or efflux of radioactive calcium ions in cells expressing a polypeptide of the invention.

Electrophysiological recordings of cell membrane currents or membrane potentials from cells expressing a polypeptide of the invention and other Kv3 subfamily members may also be used to assay for modulatory activity of a test

substance.

Preferably, electroplysological assays and/or assays comprising measuring changes in intracellular potassium ion concentration are performed on cells expressing a polypeptide of the invention and other Kv3 subfamily members.

Assays may also be carried out to identify substances which modify HIPHUM 59/60/190 expression, for example substances which up- or down- regulate expression. Such assays may be carried out for example by using antibodies for HIPHUM 59/60/190 to monitor levels of HIPHUM 59/60/190 expression. Other assays which can be used to monitor the effect of a test substance on HIPHUM 59/60/190 expression include using a reporter gene construct driven by the HIPHUM 59/60/190 regulatory sequences as the promoter sequence and monitoring for expression of the reporter polypeptide. Further possible assays could utilise membrane fractions from overexpression of HIPHUM 59/60/190 polypeptide either in *X. laevis* oocytes or cell lines such as HEK293, CHO, COS7, BHK, 3T3 and HeLa cells.

Additional control experiments may be carried out.

Suitable test substances which can be tested in the above assays include combinatorial libraries, defined chemical entities and compounds, peptide and peptide mimetics, oligonucleotides and natural product libraries, such as display (e.g. phage display libraries) and antibody products.

Typically, organic molecules will be screened, preferably small organic molecules which have a molecular weight of from 50 to 2500 daltons. Candidate products can be biomolecules including, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof. Candidate agents are obtained from a wide variety of sources including libraries of synthetic or natural compounds. Known pharmacological agents may be subjected to directed or random chemical modifications, such as acylation, alkylation, esterification, amidification, etc. to produce structural analogs.

Test substances may be used in an initial screen of, for example, 10 substances per reaction, and the substances of these batches which show inhibition or activation tested individually. Test substances may be used at a concentration of from 1nM to 1000μM, preferably from 1μM to 100μM, more preferably from 1μM

to 10μM. Preferably, the potassium channel activity of a polypeptide of the invention in response to a test substance is compared to the activity in response to depolarisation of the cell membrane. A test substance which acts as an inhibitor may produce a 50% inhibition of activity of the channel. Alternatively a test substance which acts as an activator may produce 50% of the maximal activity produced by depolarisation.

Another aspect of the present invention is the use of polynucleotides encoding the HIPHUM 59/60/190 polypeptides of the invention to identify mutations in HIPHUM 59/60/190 genes which may be implicated in human disorders. Identification of such mutations may be used to assist in diagnosis or susceptibility to such disorders and in assessing the physiology of such disorders. Polynucleotides may also be used in hybridisation studies to monitor for up- or down-regulation of HIPHUM 59/60/190 expression. Polynucleotides such as SEQ ID NO: 1 or SEQ ID NO: 3 or fragments thereof may be used to identify allelic variants, genomic DNA and species variants.

The present invention provides a method for detecting variation in the expressed products encoded by HIPHUM 59/60/190 genes. This may comprise determining the level of HIPHUM 59/60/190 expressed in cells or determining specific alterations in the expressed product. Sequences of interest for diagnostic purposes include, but are not limited to, the conserved portions as identified by sequence similarity and conservation of intron/exon structure. The diagnosis may be performed in conjunction with kindred studies to determine whether a mutation of interest co-segregates with disease phenotype in a family.

Diagnostic procedures may be performed on polynucleotides isolated from an individual or alternatively, may be performed *in situ* directly upon tissue sections (fixed and/or frozen) of patient tissue obtained from biopsies or resections, such that no nucleic acid purification is necessary. Appropriate procedures are described in, for example, Nuovo, G.J., 1992, "PCR *In Situ* Hybridization: Protocols And Applications", Raven Press, NY). Such analysis techniques include, DNA or RNA blotting analyses, single stranded conformational polymorphism analyses, *in situ* hybridization assays, and polymerase chain reaction analyses. Such analyses may reveal both quantitative aspects of the expression pattern of a HIPHUM 59/60/190,

and qualitative aspects of HIPHUM 59/60/190 expression and/or composition.

Alternative diagnostic methods for the detection of HIPHUM 59/60/190 nucleic acid molecules may involve their amplification, e.g. by PCR (the experimental embodiment set forth in U.S. Patent No. 4,683,202), ligase chain reaction (Barany, 1991, Proc. Natl. Acad. Sci. USA 88:189-193), self sustained sequence replication (Guatelli *et al.*, 1990, Proc. Natl. Acad. Sci. USA 87:1874-1878), transcriptional amplification system (Kwoh *et al.*, 1989, Proc. Natl. Acad. Sci. 15 USA 86:1173-1177), Q-Beta Replicase (Lizardi *et al.*, 1988, Bio/Technology 6:1197) or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

Particularly suitable diagnostic methods are chip-based DNA technologies such as those described by Hacia *et al.*, 1996, Nature Genetics 14:441-447 and Shoemaker *et al.*, 1996, Nature Genetics 14:450-456. Briefly, these techniques involve quantitative methods for analyzing large numbers of nucleic acid sequence targets rapidly and accurately. By tagging with oligonucleotides or using fixed probe arrays, one can employ chip technology to segregate target molecules as high density arrays and screen these molecules on the basis of hybridization.

Following detection, the results seen in a given patient may be compared with a statistically significant reference group of normal patients and patients that have HIPHUM 59/60/190 related pathologies. In this way, it is possible to correlate the amount or kind of HIPHUM 59/60/190 encoded product detected with various clinical states or predisposition to clinical states.

Another aspect of the present invention is the use of the substances that have been identified by screening techniques referred to above in the treatment of disease states, which are responsive to regulation of voltage-gated potassium channel activity. The treatment may be therapeutic or prophylactic. The condition of a patient suffering from such a disease state can thus be improved.

In particular, such substances may be used in the treatment of epilepsy, juvenile myoclonic epilepsy (JME), temporal lobe epilepsy (TLE), seizure disorders, sleep disorders such as insomnia, hypersomnia, parasomnia, sleep apnea syndromes

and stupor, pain states such as acute postoperative pain, psychogenic pain syndromes, pain from cancer, glossopharyngeal neuralgia, inflammatory pain, neuropathic pain, migraine, trigeminal neuralgia, headache and tension headache, neurodegenerative diseases such as Alzheimer's disease, Huntington's disease, Parkinson's disease, palsies and paralysis, pyschiatric disorders such as anxiety, depression, bipolar disorder, schizophrenia, paranoid psychoses and thyroid disorders such as euthyroid sick syndrome, hyperthyroidism, hypothyroidism, simple goiter and thyroiditis.

Additional disease states that may be treated include agnosia, akathisia, amnesias, anxiety disorders, bipolar disease, coma, delirium, dyskinesia, Friedreich ataxia, idiopathic orthostatic hypotension, Shy-Drager syndrome, post traumatic stress disorder, tardive dyskihesia and tremor.

Substances identified according to the screening methods outlined above may be formulated with standard pharmaceutically acceptable carriers and/or excipients as is routine in the pharmaceutical art. For example, a suitable substance may be dissolved in physiological saline or water for injections. The exact nature of a formulation will depend upon several factors including the particular substance to be administered and the desired route of administration. Suitable types of formulation are fully described in Remington's Pharmaceutical Sciences, Mack Publishing Company, Eastern Pennsylvania, 17th Ed. 1985, the disclosure of which is included herein of its entirety by way of reference.

The substances may be administered by enteral or parenteral routes such as via oral, buccal, anal, pulmonary, intravenous, intra-arterial, intramuscular, intraperitoneal, topical or other appropriate administration routes.

A therapeutically effective amount of a modulator is administered to a patient. The dose of a modulator may be determined according to various parameters, especially according to the substance used; the age, weight and condition of the patient to be treated; the route of administration; and the required regimen. A physician will be able to determine the required route of administration and dosage for any particular patient. A typical daily dose is from about 0.1 to 50 mg per kg of body weight, according to the activity of the specific modulator, the age, weight and conditions of the subject to be treated, the type and severity of the degeneration and

the frequency and route of administration. Preferably, daily dosage levels are from 5 mg to 2 g.

Nucleic acid encoding HIPHUM 59/60/190 or a variant thereof which inhibits or enhances HIPHUM 59/60/190 activity or antisense nucleic acid may be administered to the mammal. Nucleic acid, such as RNA or DNA, and preferably, DNA, is provided in the form of a vector, such as the polynucleotides described above, which may be expressed in the cells of the mammal.

Nucleic acid administered to the mammal for gene therapy may encode functional HIPHUM 59/60/190 or a variant thereof with an impaired function such as a dominant negative mutant that disrupts the function of the whole voltage-gated potassium channel.

Nucleic acid encoding the polypeptide may be administered by any available technique. For example, the nucleic acid may be introduced by needle injection, preferably intradermally, subcutaneously or intramuscularly. Alternatively, the nucleic acid may be delivered directly across the skin using a nucleic acid delivery device such as particle-mediated gene delivery. The nucleic acid may be administered topically to the skin, or to mucosal surfaces for example by intranasal, oral, intravaginal or intrarectal administration.

Uptake of nucleic acid constructs may be enhanced by several known transfection techniques, for example those including the use of transfection agents. Examples of these agents includes cationic agents, for example, calcium phosphate and DEAE-Dextran and lipofectants, for example, lipofectam and transfectam. The dosage of the nucleic acid to be administered can be altered. Typically the nucleic acid is administered in the range of 1pg to 1mg, preferably to 1pg to 10μg nucleic acid for particle mediated gene delivery and 10μg to 1mg for other routes.

The following Examples illustrate the invention.

Example 1: Characterisation of the sequence

A voltage-gated potassium channel, designated as HIPHUM 59/60/190 has been identified. The nucleotide and amino acid sequences of HIPHUM 59/60/190 have been determined and two splice variants have been identified. The nucleotide and amino acid sequences of the two variants are set out below in SEQ ID NOs: 1 to

4. Suitable primers and probes were designed and used to analyse tissue expression. HIPHUM 59/60/190 was found to be primarily expressed in brain (whole brain, fetal brain, cerebral cortex, cerebellum, hypothalamus) and thyroid.

The chromosomal localization was also mapped. Human HIPHUM 59/60/190 has been mapped to 12q14-q15.

Example 2: Screening for substances which exhibit protein modulating activity

Mammalian cells, such as HEK293, CHO, COS, BHK, 3T3 or HeLa cells, or *Xenopus* oocytes over-expressing a polypeptide of the invention together with one or more appropriate voltage-gated potassium channel subunit are generated for use in the assay. 96 and 384 well plate, high throughput screens (HTS) are employed using fluorescence based K^+/Rb^+ indicator molecules or voltage sensitive indicator molecules. Secondary screening involves electrophysiological assays utilising two electrodes, voltage clamp or patch clamp technology. Tertiary screens involve the study of modulators in rat and mouse models of disease relevant to the target.

A brief screening assay protocol based on a K^+/Rb^+ binding fluorescent dye is as follows. Mammalian cells stably over-expressing the polypeptide of the invention together with appropriate voltage-gated potassium channel subunit proteins for making a potassium channel are cultured in 96 or 384 well plates. One T225cm ^{3} flask is sufficient for setting up ten 96 well plates with a volume of 100ml cell culture medium in each well. These plates are set up the night before each assay run. The culture media is removed and 100ml of assay buffer (125mM Choline chloride, 50mM HEPES, 5.5mM Glucose, 0.8mM $MgSO_4$, 5mM KCl, pH 7.4) is added. The cells are then loaded with the K^+/Rb^+ indicator dye of choice for 30 minutes. The test compounds are added to the wells and pre-incubated for a period of 10 minutes. The channel is activated by depolarising the cell membrane. Modulation of the activity of a polypeptide of the invention results in either an increase or a decrease in the activity of the channel and the change in intracellular K^+/Rb^+ can be measured directly in a Fluorescence Imaging Plate Reader (FLIPR; Molecular Devices).

A typical electropysiology protocol using electrophysiology in oocytes expressing HIPHUM 59/60/190 is as follows. HIPHUM 59/60/190 is expressed in *Xenopus laevis* oocytes either:

i) by injection of plasmid DNA that allows the expression of the ion channel cDNA or gene by virtue of an upstream promoter (for example the CMV promoter), or preferably

ii) by injection of *in vitro* transcribed, m'G(5')pp(5')GTP-capped, complementary RNA synthesised from the ion channel cDNA by virtue of an upstream Sp6, T3 or T7 promoter and Sp6, T3 or T7 RNA polymerase.

Typically, 20-50ng of plasmid DNA or cRNA is injected per oocyte and whole-cell currents are recorded using two-microelectrode voltage-clamp (Geneclamp amplifier, Axon instruments Inc.) 1 to 7 days post-injection. Typical microelectrodes have a resistance of 0.5 to 2MΩ and are filed with 3M KCl. Oocytes are voltage-clamped at a set holding membrane potential (for example, between -100mV to -80mV) in ND96 solution (superfused at 2ml per min.) and depolarising voltage pulses are applied to activate the channels.

Potassium currents elicited by these voltage pulses are recorded. Voltage-protocols can be generated using pCLAMP8 software (Axon Instruments) and a P/N leak subtraction protocol is used throughout (to remove artefacts generated by non-specific 'leak' current across the membrane). In these experiments the effects of a test compound on current mediated by the channel is studied by inclusion of the compound in the extracellular buffer which is superfused across the oocyte.

A typical electrophysiology assay using mammalian cells expressing a polypeptide of the invention is as follows.

Cells are grown on a glass coverslip, placed into a recording chamber (0.5ml volume) and superfused with an extracellular recording solution at 2 ml min^{-1}. Drugs are applied either via addition to the bath perfusate, or alternatively using a rapid perfusion system which consists of a series of reservoirs connected to a small microfil tube that is placed in close proximity to the voltage-clamped cell. Whole-cell currents are recorded using an Axopatch 200B amplifier (Axon Instruments) or other voltage-clamp amplifier (e.g. HEKA), using standard electrophysiological methods (Hamill *et al.,* 1981). Patch pipettes are fabricated from 1.5mm outside

diameter borosilicate capillary glass (Clark Electromedical) using a micropipette puller (Sutter model P97), and fire polished (Narishige Microforge) to give final tip resistances of 2-4MΩ. A silver/silver chloride pellet is used as the bath reference electrode and the potential difference between this and the recording electrode will be adjusted for zero current flow before seal formation. Cells are visualised using a Diaphot200 inverted microscope (Nikon) with modulation contrast optics at a final magnification of x400. High resistance seals (1-10GΩ) between pipette and neuronal cell membranes are achieved by gentle suction, and the 'whole cell' configuration attained by applying further suction.

Cells are patch-clamped in an extracellular buffer containing 140mM NaCl, 4.7mM KCl, 1.2mM MgCl2, 1mM CaCl2, 11mM glucose, 5mM HEPES (titrated with NaOH to pH 7.4 at 25°C) using microelectrode pipettes containing 130mM KCl, 3mM NaCl, 1mM MgCl2, 5mM K-EGTA (ethylene glycol-bis(β -aminoethyl ester) N,N,N',N-tetra acetic acid, K salt), 10mM HEPES, 5mM Glucose, 3mM Mg-ATP (pH7.3 at 25°C). Patch electrodes should have resistances of 2 to 6MΩ when filled with the pipette-filling solution. Cells are voltage-clamped at a set holding membrane potential (for example, between -100mV to -80mV) and depolarising voltage pulses are applied to activate the channels.

Voltage command protocols are generated, and current records stored, via a digidata 1200 analog/digital interface (Axon Instruments) controlled by microcomputer (Hewlett Packard Kayak XA) using pCLAMP8 Clampex software (Axon Instruments). Signals are prefiltered at 5kHz bandwidth and sampled at 20kHz. Capacitance transients and series resistance errors are compensated for (80-85%) using the amplifier circuitry, and linear leakage currents subtracted using an on-line 'P-4' procedure provided by the commercial software package.

Data are analysed using pCLAMP8/Clampfit (Axon Instruments), ORIGIN (MicroCal) and DAISI data handling and graphical presentation software packages. Results can be presented as either arithmetic mean ± s.e mean or geometric mean with 95% confidence limits. Statistical comparisons are made using paired or unpaired Student's t-test and considered of significance when $P<0.05$.

Channel activity is monitored in the presence and absence of a test substance

and modulation of channel activity by the test substance is compared in the presence and absence of the test substance to determine whether the test substance is an agonist or antagonist of the voltage-gated potassium channel.

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CLAIMS

1. An isolated voltage-gated potassium channel polypeptide comprising

(i) the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4; or

(ii) a variant thereof which capable of forming a channel which can be activated by depolarisation of the cell membrane potential above the reversal potential for K^+ (E_K); or

(iii) a fragment of (i) or (ii) which capable of forming a channel which can be activated by depolarisation of the cell membrane potential above the reversal potential for K^+ (E_K).

2. A polypeptide according to claim 1 wherein the variant (ii) has at least 98% identity to the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4.

3. A polynucleotide encoding a polypeptide according to claim 1 or 2.

4. A polynucleotide according to claim 3 which is a cDNA sequence.

5. A polynucleotide encoding a voltage-gated potassium channel polypeptide which is capable of forming a channel which can be activated by depolarisation of the cell membrane potential above the reversal potential K^+ (E_K), which polynucleotide comprises:

(a) the nucleic acid sequence of SEQ ID NO: 1 or SEQ ID NO: 3 and/or a sequence complementary thereto;

(b) a sequence which hybridises under stringent conditions to a sequence as defined in (a);

(c) a sequence that is degenerate as a result of the genetic code to a sequence as defined in (a) or (b); or

(d) a sequence having at least 98% identity to a sequence as defined in (a), (b) or (c).

6. An expression vector comprising a polynucleotide according to any one of claims 3 to 5.

7. A host cell comprising an expression vector according to claim 6.

8. An antibody specific for a polypeptide according to claim 1 or 2.

9. A method for the identification of a substance that modulates voltage-gated potassium channel activity and/or expression, which method comprises:

(i) contacting a test substance and a polypeptide according to claim 1 or

2, a polynucleotide according to any one of claims 3 to 5, an expression vector according to claim 6 or a host cell according to claim 7, and

(ii) determining the effect of the test substance on the activity and/or expression of the said polypeptide or the polypeptide encoded by said polynucleotide, thereby to determine whether the test substance modulates voltage-gated potassium channel activity and/or expression.

10. A method according to claim 9 wherein the polypeptide is expressed in a cell.

11. A method according to claim 10 wherein the cell expresses other Kv3 subfamily members.

12. A method according to any one of claims 9 to 11 wherein step (ii) comprises monitoring any voltage-gated potassium channel activity.

13. A substance which modulates voltage-gated potassium channel activity and which is identifiable by a method according to any one of claims 9 to 12.

14. A method of treating a subject having a disorder that is responsive to voltage-gated potassium channel modulation, which method comprises administering to said subject an effective amount of a substance according to claim 13.

15. A method according to claim 14 wherein the disorder is selected from epilepsy, juvenile myoclonic epilepsy (JME), temporal lobe epilepsy (TLE), seizure disorders, sleep disorders such as insomnia, hypersomnia, parasomnia, sleep apnea syndromes and stupor, pain states such as acute postoperative pain, psychogenic pain syndromes, pain from cancer, glossopharyngeal neuralgia, inflammatory pain, neuropathic pain, migraine, trigeminal neuralgia, headache and tension headache, neurodegenerative diseases such as Alzheimer's disease, Huntington's disease, Parkinson's disease, palsies and paralysis, pyschiatric disorders such as anxiety, depression, bipolar disorder, schizophrenia, paranoid psychoses and thyroid disorders such as euthyroid sick syndrome, hyperthyroidism, hypothyroidism, simple goiter and thyroiditis.

16. Use of a substance as defined in claim 13 in the manufacture of a medicament for treatment or prophylaxis of a disorder that is responsive to

stimulation or modulation of voltage-gated potassium channel activity.

17. A use according to claim 16 wherein the disorder is selected from epilepsy, juvenile myoclonic epilepsy (JME), temporal lobe epilepsy (TLE), seizure disorders, sleep disorders such as insomnia, hypersomnia, parasomnia, sleep apnea syndromes and stupor, pain states such as acute postoperative pain, psychogenic pain syndromes, pain from cancer, glossopharyngeal neuralgia, inflammatory pain, neuropathic pain, migraine, trigeminal neuralgia, headache and tension headache, neurodegenerative diseases such as Alzheimer's disease, Huntington's disease, Parkinson's disease, palsies and paralysis, pyschiatric disorders such as anxiety, depression, bipolar disorder, schizophrenia, paranoid psychoses and thyroid disorders such as euthyroid sick syndrome, hyperthyroidism, hypothyroidism, simple goiter and thyroiditis.

18. A method of producing a polypeptide according to claim 1 or 2, which method comprises maintaining a host cell as defined in claim 7 under conditions suitable for obtaining expression of the polypeptide and isolating the said polypeptide.

THE PATENT OFFICE

INVESTOR IN PEOPLE

Application No: GB 0025677.6
Claims searched: 1-12, 18

40

Examiner: Dr Jeremy Kaye
Date of search: 19 June 2002

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.T):

Int Cl (Ed.7):

Other: Online: EPODOC, WPI, PAJ, BIOSIS, CAPLUS, EMBASE, MEDLINE, SCISEARCH, BLASTp, BLASTn

Documents considered to be relevant:

Category	Identity of document and relevant passage	Relevant to claims
E, X	WO 02/04520 A2 (INCYTE GENOMICS) SEQ ID NO:18, Figs pp.29/87-30-87.	1-12, 18
X	FEBS Letts., Vol.288, 1991, Luneau, C. et al., "Shaw-like rat brain potassium channel...", pp.163-167 & related Genbank Accession number M59211	1-12, 18
X	P.N.A.S., Vol.87, 1990, McCormack, T. et al., "Molecular cloning of a member...", pp.5227-5231 & related Genbank Accession Number M34052, "Rat K+ channel protein..."	1-12, 18

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
		E	Patent document published on or after, but with priority date earlier than, the filing date of this application.
&	Member of the same patent family		